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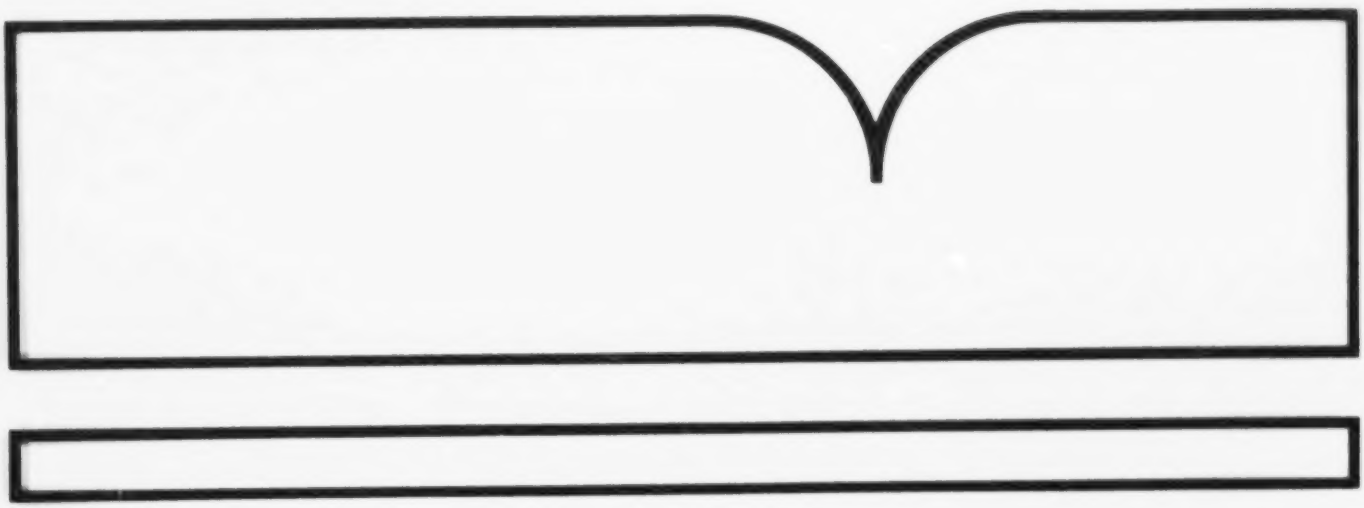

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16. ABSTRACT

A comparison of the concentration-response effects of inhaled ozone (O_3) in different species of laboratory animals was made in order to better understand the influence of the choice of species in inhalation studies of this gas. The effect of 4 hr exposure to ozone (O_3) at concentrations of 0.0, 0.2, 0.5, 1.0, and 2.0 ppm was determined in rabbits, guinea pigs, rats, hamsters and mice. Lavage fluid protein (LFP) accumulation 18 hr after exposure was used as the indicator of O_3-induced pulmonary edema. All species had similar basal levels of LFP (250-350 µg/ml) when a volume of saline which approximated the total lung capacity was used for lavage of the collapsed lungs. Exponential dose-response curves were seen in all species except guinea pigs, which showed significant increases in LFP at low O_3 concentrations (0.2 ppm) and a leveling off of response at the higher O_3 levels. Other species usually showed significant elevations in LFP only at concentrations greater than 0.5 ppm. Recovery of lavage fluid as a percent of the amount of injected saline varied significantly between species, and between O_3 concentrations; higher O_3 levels causing lower recovery. The time course of LFP accumulation also appeared to vary according to the species.

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SPECIES COMPARISON OF ACUTE INHALATION TOXICITY OF OZONE AND PHOSGENE

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A comparison of the concentration-response effects of inhaled ozone (O_3) and phosgene ($COCl_2$) in different species of laboratory animals was made in order to better understand the influence of the choice of species in inhalation toxicity studies. The effect of 4-h exposures to ozone at concentrations of 0.2, 0.5, 1.0, and 2.0 ppm, and to $COCl_2$ and 0.1, 0.2, 0.5, and 1.0 ppm was determined in rabbits, guinea pigs, rats, hamsters, and mice. Lavage fluid protein (LFP) accumulation 18–20 h after exposure was used as the indicator of O_3- and $COCl_2$-induced pulmonary edema. All species had similar basal levels of LFP (250–350 mg/ml) when a volume of saline that approximated the total lung capacity was used to lavage the collapsed lungs. Ozone effects were most marked in guinea pigs, which showed significant effects at 0.2 ppm and above. Mice, hamsters, and rats showed effects at 1.0 ppm O_3 and above, while rabbits responded only at 2.0 ppm O_3. Phosgene similarly affected mice, hamsters, and rats at 0.2 ppm and above, while guinea pigs and rabbits were affected at 0.5 ppm and above. Percent recovery of lavage fluid varied significantly between species, guinea pigs having lower recovery than other species with both gases. Lavage fluid recovery was lower following exposure to higher levels of O_3 but not $COCl_2$. Results of this study indicate that significant species differences are seen in the response to low levels of O_3 and $COCl_2$. These differences do not appear to be related in a simple manner to body weight.

INTRODUCTION

Qualitative differences in sensitivity to ozone (O_3) between animal species have been described (Jones et al., 1970; Chow et al., 1975; Drew et al., 1976, Cavender et al., 1978; Mustafa et al., 1982). However, differences in techniques used in different laboratories make rigorous quantitative comparisons impossible (Graham and Hatch, 1984). The present study investigates the hypothesis that quantitative concentration-effect data in several species might provide the basis for future mechanism studies, as well as more rigorous extrapolations of animal toxicity data to humans. We have examined the concentration-effect relationship of two inhaled

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gases, O_3 and phosgene ($COCl_2$), in five commonly used laboratory animals: rabbits, guinea pigs, rats, hamsters, and mice. Ozone is a ubiquitous air pollutant, and $COCl_2$ has also been detected in ambient air (Singh, 1976). Both gases share similar toxic effects when inhaled, including pulmonary edema, increased susceptibility to bacterial infection, prolonged pentobarbital-induced sleep time, and characteristic changes in pulmonary function and biochemistry (Alarie, 1973; Gardner, 1982; Franch and Hatch, 1986). Both O_3 and $COCl_2$ are highly electronegative gases with high reactivity with constituents of tissues compared to their solubility or reactivity with water (Nash and Pattle, 1971; Miller et al., 1985). Therefore, uptake of these gases would be expected to be the greatest in the respiratory-tract tissues, and factors such as minute ventilation should be of prime importance in determining the dose to these tissues. On this basis some species differences in response to inhaled compounds might be expected. For example, according to published allometric relationships of animals at rest (Stahl, 1967), mice should inhale about three times more air (and presumably O_3 or $COCl_2$ if present in the air) per body weight than rabbits. Also, differences in lung antioxidant concentrations between species have been reported that could influence the sensitivity to these pollutants (Slade et al., 1985, White and Repine, 1985).

Previous work in our laboratory has demonstrated that lavage fluid protein (LFP) accumulation is a quantitative index of low-level edema that should be applicable to any animal species if the quantity of saline used for lavage is indexed to the lung volume of the animal (Selgrade et al., 1981; Alpert et al., 1971). The simplicity of this assay system suggested the need for a preliminary assessment of species sensitivity of resting laboratory animals to O_3 and $COCl_2$, which could provide guidance to future studies that could relate differences in toxicity to biochemical and physiological parameters in each species. The inclusion of the two gases was expected to increase the possibility of detecting patterns of species sensitivity.

METHODS

Animals

Swiss albino mice (CRL:CD-1, 19–25 g), Hartley strain guinea pigs (314–522 g), and Sprague-Dawley rats (CD, 280–350 g) were obtained from Charles River Breeding Labs., Kingston, N.Y. New Zealand White rabbits (1.7–2.5 kg) were purchased from Hazelton/Dutchland Laboratories Inc., Denver Pa., and Golden Syrian hamsters (94–107 g) from Engle Laboratory Animals, Farmersberg, Ind. They were housed in termperature- and humidity-controlled rooms with a 12-h light/dark cycle and fed standard diets obtained from Allied Mills, Chicago, Ill., or Ralston Purina Co., St. Louis, Mo. (rat diet). All animals were specific-pathogen-free, and were

used within 2 wk of arrival. Since preliminary studies with rats, mice, and guinea pigs indicated no difference between male and female animals, all animals studied were males except mice. Because of fighting among male mice, females were used.

Exposures

Animals were exposed in individual stainless-steel wire-mesh modules placed in a 11.3-ft^3 Rochester-type chamber (Hinners et al., 1968). Control animals were caged identically and exposed to filtered room air concurrent with the exposure. Exposures to O_3 and $COCl_2$ were performed on the same shipments of animals during the same week. A given experiment consisted of four animals from a given species exposed per group in one of five concentrations of gas plus air controls.

Ozone was generated from O_2 using a silent-arc-discharge O_3 generator (OREC, Phoenix, Ariz.) and was mixed with filtered room air, which was introduced into the chamber at a rate of 1 chamber volume/min. A mass flow controller was used to control the amount of O_3 entering the chamber. The concentration of O_3 was monitored continuously using chemiluminescent O_3 analyzers (Bendix, Lewisburg, W. Va.), which were calibrated biweekly using a Dasibi transfer standard, which was referenced quarterly to a primary ultraviolet O_3 standard (National Technical Information Service, 1979a,b). Chamber concentrations of O_3 in the studies reported here deviated less than 6% from the stated concentration.

Phosgene, obtained as a mixture in nitrogen (Matheson Gas Products, Morrow, Ga.), was mixed with filtered room air before the metering orifice of the chamber and introduced into the chamber airstream, which had a flow rate of 1 chamber volume/min. A Tylan mass flow controller was used to meter the $COCl_2$ into the airstream, and the chamber air was sampled every 10 min. Phosgene concentrations in the chamber were determined with a Hewlett Packard 11831 gas chromatograph containing a 6 ft × $\frac{1}{8}$ in. Teflon column filled with Chromosil 310 packing (Supelco Incl., Bellefonte, Pa.) operating at 70°C (Esposito et al., 1977). Methane (5%) in argon was used as the mobile phase at a flow rate of 43 cm^3/min. Phosgene was detected by electron capture. The gas chromatograph was calibrated at least bimonthly with a permeation tube containing liquid $COCl_2$ (Analytical Instrument Development Inc., Avondale, Pa.). A Wilks model 80 infrared analyzer was used as a second check of the $COCl_2$ concentration. Actual chamber concentrations always deviated less than 8.2%, and usually ranged from 2 to 6% of the stated concentration.

Lavage of Animals and Protein Determination

Our previous studies showed that maximal LFP accumulation occurred about 18 h after the termination of a 3-h exposure to minimally effective concentrations of NO_2 or O_3 (Selgrade et al., 1981; Hu et al., 1982). Other studies (W. D. Currie and G. E. Hatch, 1986, unpublished) indicated that low-

level (<0.5 ppm, 4 h) $COCl_2$ exposures also showed maximal LFP 18–24 h postexposure. Since our purpose was to examine the lower portion of the concentration-response curves at a time that would indicate species differences in tissue damage rather than repair, we lavaged animals 18–20 h following the 4-h exposure to the gases.

Animals were anesthetized by ip injection of Nembutal (2 ml/kg bw, diluted 1:5 with saline) and were exsanguinated through the abdominal aorta, and the lungs were collapsed by puncturing the diaphragm. A cannula was inserted into the trachea and secured with a ligature, and a volume of saline warmed to 37°C and equal to 35 ml/kg body weight was injected. This saline was immediately withdrawn and reinjected an additional two times. It was then withdrawn and kept on ice until centrifuged at 700 × g to remove cells. Supernatant fractions of the lavage fluid were stored at −20°C until assayed for protein according to the method of Lowry et al. (1951). The volume of saline used for injection was determined from allometric equations published by Takezawa et al. (1980) and equaled about 90% of the total lung capacity in all species. The volume of lavage fluid recovered from each animal was recorded. Separate experiments indicated that percent recovery of lavage fluid did not of itself significantly alter the LFP: the protein concentration of the first and second halves of the recovered lavage fluid differed by less than 8%.

Statistical Analysis

Pooled data from two experiments per species was log-transformed to achieve homogeneity of variances for regression analyses. Thus, analyses were performed on log-transformed data, although tables show means and SE of nontransformed data. Univariate analyses of variance were also performed so that concentration-response data could be evaluated by the methods of Williams (1972) and Dunnett (1955).

RESULTS

Species Comparison of O_3 Concentration-Response

A summary of the lavage fluid protein concentrations resulting from the species comparison studies with O_3 is given in Table 1. Analysis of variance indicated no species-related differences in the basal lavage fluid protein concentrations, which ranged from mean levels of about 200 to 400 mg/ml. All of the experiments showed a definite trend of increasing LFP with increasing O_3 concentration. Williams's test indicated that guinea pigs responded significantly at all concentrations higher than 0.2 ppm, while rats, hamsters, and mice showed significant effects at 1.0 ppm and above. Rabbits showed significant effects only at 2.0 ppm. Application of Dunnett's test indicated the same values as being statistically significant as did Williams's test.

TABLE 1. Ozone Effects on Lavage Fluid Protein: Species Comparison[a]

Species	Air	Ozone exposure			
		0.2 ppm	0.5 ppm	1.0 ppm	2.0 ppm
Mouse	363 ± 37 (8)	405 ± 30 (6)	464 ± 29 (8)[b]	648 ± 58 (8)	1188 ± 142 (7)
Hamster	366 ± 21 (8)	363 ± 25 (8)	294 ± 10 (8)	557 ± 88 (8)[b]	945 ± 102 (8)
Rat	243 ± 28 (8)	362 ± 63 (8)	356 ± 68 (7)	528 ± 37 (8)[b]	1839 ± 201 (8)
Guinea pig	329 ± 24 (8)	470 ± 32 (8)[b]	645 ± 58 (7)	1006 ± 12 (7)	1139 ± 61 (8)
Rabbit	317 ± 23 (12)	386 ± 37 (12)	362 ± 29 (12)	461 ± 62 (12)	849 ± 133 (12)[b]

[a]Animals were exposed to O_3 for 4 h and lavaged with saline 18–20 h later. Mean ± SE are given. Parentheses indicate number of animals.

[b]Lowest level of exposure producing a significantly elevated ($p < 0.05$) level of protein, Williams's (1972) test.

Fitting of the concentration-response curves yielded the common slope relationship, $\log[\text{protein}] = a + 0.77[O_3]$, where the intercept a varied between 5.4 and 5.6, depending on the species. This regression model explained 87% of the variation about the weighted means for the non-guinea pig data. Guinea pigs did not fit into the exponential model because of the high LFP accumulation seen at lower O_3 concentrations and the apparent leveling off at higher O_3 concentrations.

Species Comparison of $COCl_2$ Concentration-Response

Concentrations of $COCl_2$ chosen for this study were lower than those of O_3 because preliminary evidence suggested a difference in the toxicity of the two gases. Table 2 shows a summary of the results of $COCl_2$ exposure on LFP accumulation. As with the O_3 studies, basal LFP concentrations were not significantly different between species. Williams's test indicated

TABLE 2. Phosgene Effects on Lavage Fluid Protein: Species Comparison[a]

Species	Air	Phosgene exposure			
		0.1 ppm	0.2 ppm	0.5 ppm	1.0 ppm
Mouse	292 ± 18 (8)	302 ± 21 (8)	941 ± 105 (8)[b]	1302 ± 149 (8)	2168 ± 167 (8)
Hamster	319 ± 6 (8)	347 ± 14 (8)	520 ± 63 (8)[b]	1289 ± 92 (8)	3035 ± 111 (8)
Rat	340 ± 38 (8)	258 ± 18 (8)[b]	506 ± 54 (8)[c]	1642 ± 116 (8)	2471 ± 125 (8)
Guinea pig	305 ± 19 (8)	228 ± 47 (8)	407 ± 75 (7)	524 ± 47 (8)[b]	1212 ± 149 (8)
Rabbit	292 ± 11 (8)	309 ± 20 (8)	346 ± 26 (8)	517 ± 68 (8)[b]	855 ± 71 (8)

[a]Animals were exposed to $COCl_2$ for 4 h and lavaged with saline 18–20 h later. Mean ± SE are given. Parentheses indicate number of animals.

[b]Lowest level of exposure producing a significantly altered ($p < 0.05$) level of protein, Williams's (1972) test.

[c]Lowest level of exposure producing a significantly altered ($p < 0.05$) level of protein, Dunnett's (1955) test (rats only).

that there was a significant response in rats at the lowest $COCl_2$ concentration (0.1 ppm). However, since the observed mean LFP was decreased rather than increased at this concentration, the effect appeared to be a statistical artifact. Dunnett's test indicated no effect at 0.1 ppm $COCl_2$ in rats, and a significant effect at 0.2 ppm and higher. Williams's test and Dunnett's test showed similar results for the other species. Mice and hamsters showed significant effects above 0.2 ppm, and guinea pigs and rabbits responded significantly at 0.5 ppm $COCl_2$ and above.

In the log-linear regression of protein on $COCl_2$ concentration, again, as in the case with O_3, no single common slope relationship could adequately fit the response pattern for all species. One common regression model fit the guinea pig and rabbit data and another fit the data for hamsters, mice, and rats. For guinea pigs and rabbits, the common slope was estimated as 1.20 with intercepts of 5.68 and 5.61, respectively. For hamsters, mice and rats, the estimated slope was 2.31 with intercepts of 5.74, 5.73, and 5.55, respectively. The guinea pig–rabbit model explained

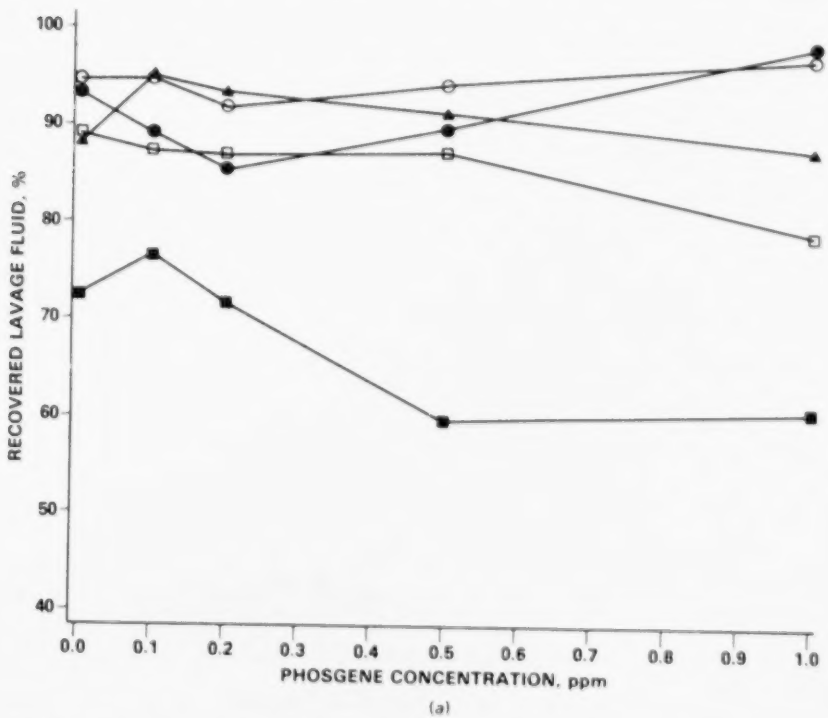
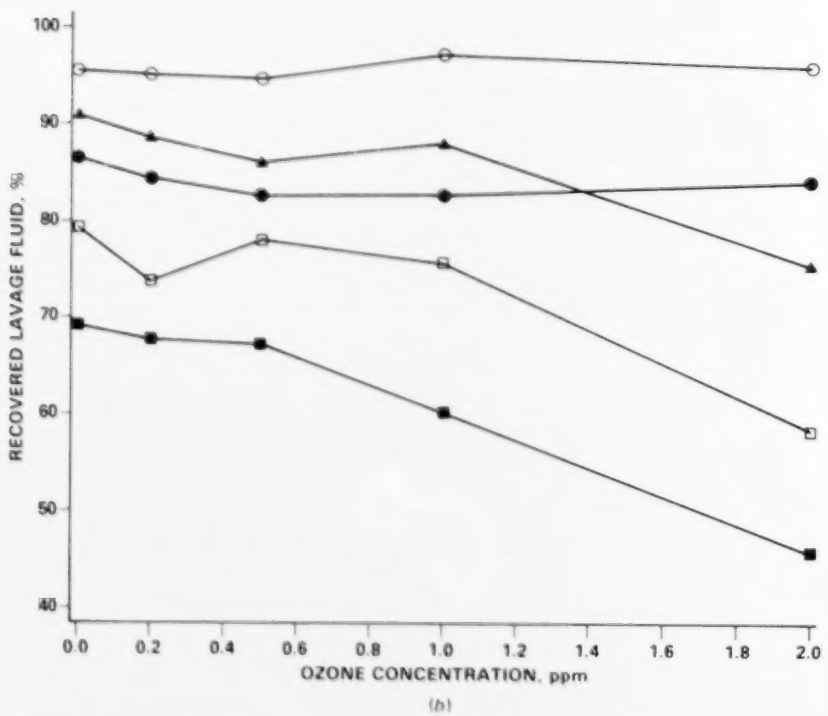


FIGURE 1. Comparison of laboratory animal species in the percent recovery of lavage fluid after exposure to different concentrations of (*a*) $COCl_2$. Symbols for the animal species are as follows: rabbits □, guinea pigs ■, rats ▲, hamsters ○, and mice ●. Asterisks denote significant differences from nonexposed group ($p < 0.5$) from application of Williams's (1972) test.

nearly 90% of the variation about the weighted means, and the hamster-mouse-rat model explained 96%.

Species Differences in Recovery of Lavage Fluid

The lavage fluid recovered, expressed as a percentage of the amount injected, appeared to vary according to the species (Fig. 1). Recovery of lavage fluid following O_3 ranged from as low as 45% in guinea pigs to as high as 97% in hamsters. Averaging percent fluid recovered over all concentration groups led to the following ranking among species: guinea pigs < rabbits < mice, rats < hamsters. Comparing all possible pairs of means (Kramer, 1957) with an overall 5% type I error rate indicated that all species were significantly different from each other except mice and rats. Rabbits, guinea pigs, and rats appeared to show decreasing lavage fluid recovery with increasing O_3 concentration, although this effect was only significant in the latter two species. Guinea pigs had the largest decrease in percent recovery due to O_3 exposure (about 20%).



(*b*)

FIGURE 1. (*Continued*) (*b*) O_3.

Following exposure to $COCl_2$, recovery of lavage fluid ranged from as low as 60% in guinea pigs to as high as 98% in mice. Averaging over all concentration groups produced the same ranking of means among species as was seen for O_3. Statistically, guinea pigs had significantly lower recovery than other species, and rabbits had lower recovery than hamsters. Recovery following $COCl_2$ exposure appeared not to be dependent on the gas concentration as was the case with O_3.

Comparison of O_3 Effects in Male and Female Rats

During the course of these experiments, results from other studies that compared the basal lung concentrations of α-tocopherol, ascorbic acid, and nonprotein sulfhydryls indicated that female rats had 60% higher lung α-tocopherol concentrations, although males and females of other species studied here had similar levels of α-tocopherol, ascorbic acid, and nonprotein sulfhydryls (Slade et al., 1985). Therefore, an additional study was performed in which male and female rats were compared in their response to 0.2, 0.5, 1.0, and 2.0 ppm O_3. Results indicated that the concentration-effect curves for both sexes were virtually superimposable. At the 2.0 ppm O_3 level, there was a 17% greater response in the male animals, which was not statistically different from the response in females (data not shown).

DISCUSSION

The present study sought to determine whether species differences in the toxicity of inhaled O_3 and $COCl_2$ could be detected in laboratory animals. The study focused on the lower end of the concentration response curve for pulmonary edema. The measurement of lung lavage fluid protein appeared to be equally applicable to all the species studied because the volume of saline injected for lavage was indexed to the approximate lung volumes. Previous studies by Alpert (1971) showed that injection of radioiodinated albumin into the bloodstream of rats exposed to O_3 resulted in leakage of the albumin into the airways, which was recoverable in lavage fluid. Significant effects were seen in the accumulation of albumin in the airways at about one-fifth of the concentrations of O_3 required to produce histologic evidence of edema. More recently, Valentini and co-workers (1983) injected ^{51}Cr-EDTA and showed similarly high sensitivity in detecting pulmonary edema as leakage of the label into the air spaces of the lung. Other studies we have performed using $COCl_2$ suggest tht the assay of LFP shows significant effects at about one-third the concentration of gas that produces a change in wet lung weight. The majority of the protein that accumulates in lavage fluid under these conditions appears to be derived from serum, as evidenced by the similarity of the banding patterns from polyacrylamide gel electrophoresis of serum and lavage fluid and the similarity of enzymes recovered (Selgrade et al., 1981; Guth and Mavis, 1985).

It was hoped at the beginning of these studies that the inclusion of two

different gases might yield similar patterns of LFP response that could be attributed to body weight or other innate differences between species. However, the five species responded quite differently to the O_3 and $COCl_2$. Guinea pigs, for example, were very sensitive to the O_3 effects but resistant to $COCl_2$ effects. Species differences also appeared to be larger with phosgene, with guinea pigs and rabbits showing a much smaller response than the other species.

Differences between species in the physiological adaptation to O_3 or $COCl_2$ exposure could be very important in determining the tissue dose at the target site for this response and, therefore, the ultimate toxicity observed. For instance, rats are known to reduce their minute ventilation when exposed to O_3 (Scheel et al., 1958), and rats and mice have the same response when exposed to formaldehyde (Chang et al., 1981; Alarie, 1973). Further studies that are now in progress in our laboratory will employ $^{18}O_3$ and $^{14}COCl_2$ to provide a determination of species differences in the dose to the lung.

Significant differences between male and female rats in the O_3 response were not observed in this study, in spite of the fact that we have shown that the female rats we used had a 60% higher lung α-tocopherol concentration than male rats (Slade et al., 1985). Goldstein and Ross (1973) also reported no sex difference in mortality when different strains of mice were exposed to higher levels of O_3 than those used here.

The percent recovery of lavage fluid appeared to vary according to species and in some cases the concentration of inhaled gas. Lowered recovery of lavage fluid may be caused by contraction of the airway musculature following exposure. Guinea pigs, which showed the lowest recovery to both O_3 and $COCl_2$, have been shown to have histamine release in the lungs following O_3 exposure (Shields and Gold, 1982). Guinea pigs are also about 10,000 times more sensitive than rats to airway resistance changes induced by aerosolized histamine (J. Wiester, 1985, personal communication). It appears, from the experiments we performed to characterize the multiple-injection lavage procedure, that the lower percent recovery of lavage fluid from rabbits and guinea pigs would have had only a small influence on the resultant LFP concentrations (a maximum of an 8% decrease in LFP due to a 50% reduction in lavage fluid recovery).

In summary, the lower ends of concentration-response effects of O_3 and $COCl_2$ on lavage fluid recovery and protein accumulation show some clear statistical differences between species. The response to these gases does not appear to be easily related to body weight or other obvious differences between the species.

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